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(54) Title: THE USE OF BACTERIAL PHAGE ASSOCIATED LYSING ENZYMES FOR THE PROPHYLACTIC AND THERAPEUTIC TREATMENT OF VARIOUS ILLNESSES

(57) Abstract: A method for the prophylactic and therapeutic treatment of bacterial infections is disclosed which comprises the treatment of an individual with an effective amount of a modified version of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, wherein the lytic enzyme is in an environment having a pH which allows for activity of said lytic enzyme; and a carrier for delivering said lytic enzyme. Additionally, a holin enzyme for puncturing the membrane may be included in the composition. This method, and composition can be used for the treatment of upper respiratory infections, skin infections, wounds, and burns, vaginal infections, eye infections, intestinal disorders and dental problems.

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gum or lozenge. Any other carrier can be used that allows for the exposure of the mouth, gums, and teeth to the lytic enzyme.

The lytic enzyme may also be incorporated in a lyophilized or dried form in tooth powder. If the lytic enzyme is to be used in an oral wash, it is preferred
5 that the oral wash not contain any alcohol, so as to not denature the enzyme. The enzyme can also be in a liposome when mixed in with the toothpaste or oral wash. The concentrations of the enzyme units per ml of toothpaste or mouth wash can be in the range of from about 100 units/ml to about 500,000 units/ml of composition, preferably in the range of about 1000 units/ml to about 100,000
10 units/ml, and most preferably from about 10,000 to 100,000 units/ml. The pH of the toothpaste or oral wash should be in a range that allows for the optimum performance of the enzyme, while not causing any discomfort to the user of the toothpaste or oral wash.

Many modifications and variations of the present invention are possible in
15 light of the above teachings. It is, therefore, to be understood within the scope of the appended claims the invention may be protected otherwise than as specifically described.

What we claim is:

1) A method for the prophylactic or therapeutic treatment of bacterial infections, comprising:

administering an effective amount of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria to the site of the infection.

2) The method according to claim 1, further comprising delivering said lytic enzyme in a carrier suitable for delivering said lytic enzyme to the site of the infection.

3) The method according to claim 1, wherein the at least one lytic enzyme is for the treatment of *Hemophilus influenza*.

4) The method according to claim 1, wherein the at least one lytic enzyme is for the treatment of *Pseudomonas*.

5) The method according to claim 1, wherein the at least one lytic enzyme is for the treatment of *Streptococcus pneumoniae*

6) The method according to claim 1, wherein the at least one lytic enzyme is for the treatment of *Streptococcus fasciae*

7) The method according to claim 1, wherein the at least one lytic enzyme is for the treatment of *Listeria*.

8) The method according to claim 1, wherein the at least one lytic enzyme is for the treatment of *Salmonella*.

- 9) The method according to claim 1, wherein the at least one lytic enzyme is for the treatment of *E. coli*.
- 10) The method according to claim 1, wherein the at least one lytic enzyme is for the treatment of *Campylobacter*.
- 11) The method according to claim 1, wherein the at least one lytic enzyme is for the treatment of *Pseudomonas*.
- 12) The method according to claim 1, wherein the at least one lytic enzyme is for the treatment of *Streptococcus mutans*.
- 13) The method according to claim 1, wherein the at least one lytic enzyme is for the treatment of *Mycobacterium tuberculosis*.
- 14) The method according to claim 1, wherein the at least one lytic enzyme is for the treatment of *Streptococcus*.
- 15) The method according to claim 2, wherein the carrier is an inhalant.
- 16) The method according to claim 2, wherein the carrier is a topical cream
- 17) The method according to claim 2, wherein the carrier is a nasal spray.
- 18) The method according to claim 2, wherein the carrier is a syrup.
- 19) The method according to claim 2, wherein the carrier is a tablet.
- 20) The method according to claim 2, wherein the carrier is a tampon.
- 21) The method according to claim 2, wherein the carrier is a suppository.

- 22) The method according to claim 2, wherein the carrier is an eye drop solution.
- 23) The method according to claim 2, wherein the carrier is a candy.
- 24) The method according to claim 2, wherein the carrier is a chewing gum.
- 25) The method according to claim 2, wherein the carrier is a lozenge.
- 26) The method according to claim 2, wherein the carrier is a troche.
- 27) The method according to claim 2, wherein the carrier is a powder.
- 28) The method according to claim 2, wherein the carrier is an aerosol.
- 29) The method according to claim 2, wherein the carrier is a liquid.
- 30) The method according to claim 2, wherein the carrier is a liquid spray.
- 31) The method according to claim 2, wherein the carrier is a bandage.
- 32) The method according to claim 2, wherein the carrier is a toothpaste.
- 33) The method according to claim 2, wherein the carrier is an oral wash.
- 34) A method for the prophylactic and therapeutic treatment of bacterial infections of an upper respiratory tract, comprising administering a composition comprising an effective amount of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria to a mouth, throat, or nasal passage of a mammal.

35) The method according to claim 34, further comprising delivering said lytic enzyme in a carrier suitable for delivering said lytic enzyme to the mouth, the throat or the nasal passage.

36) The method according to claim 34, wherein said bacteria being treated is selected from the group consisting of *Streptococcus pneumoniae* and *Hemophilus influenza*.

37) The method according to claim 36, wherein said bacteria being treated is *Streptococcus pneumoniae*.

38) The method according to claim 36, wherein said bacteria being treated is *Hemophilus influenza*.

39) The method according to claim 34, wherein said carrier is a candy, chewing gum, lozenge, troche, tablet, a powder, an aerosol, a liquid and a liquid spray.

40) The method according to claim 34, wherein said composition further comprises a buffer that maintains pH of the composition at a range between about 4.0 and about 9.0.

41) The method according to claim 40, wherein the buffer maintains the pH of the composition at the range between about 5.5 and about 7.5.

42) The method according to claim 40, wherein said buffer comprises a reducing reagent.

43) The method according to claim 42, wherein said reducing reagent is dithiothreitol.

- 44) The method according to claim 40, wherein said buffer comprises a metal chelating reagent.
- 45) The method according to claim 44, wherein said metal chelating reagent is ethylenediaminetetracetic disodium salt.
- 46) The method according to claim 40, wherein said buffer is a citrate-phosphate buffer.
- 47) The method according to claim 34, further comprising a bactericidal or bacteriostatic agent as a preservative.
- 48) The method according to claim 34, wherein said at least one lytic enzyme is lyophilized.
- 49) The method according to claim 35, wherein said carrier further comprises a sweetener.
- 50) The method according claim 34, further comprising administering a concentration of about 100 to about 100,000 active enzyme units per milliliter of fluid in the wet environment of the nasal or oral passages.
- 51) The method according to claim 50, further comprising administering the concentration of about 100 to about 10,000 active enzyme units per milliliter of fluid in the wet environment of the nasal or oral passages.
- 52) The method according to claim 34, further comprising using said composition in the therapeutic treatment of *Streptococcus* infections.
- 53) The method according to claim 34, further comprising using said composition in the prophylactic treatment of *Streptococcus* infections.

54) The method according to claim 34, further comprising using said composition in the therapeutic treatment of *Streptococcus* infections.

55) The method according to claim 34, further comprising using said composition in the prophylactic treatment of *Hemophilus* infections.

56) The method according to claim 34, further comprising using said composition in the therapeutic treatment of *Hemophilus* infections.

57) A composition for use in the therapeutic or prophylactic treatment of a bacterial infection of an upper respiratory tract, comprising:

an effective amount of at least one lytic enzyme produced by a bacteria being infected with a bacteriophage specific for said bacteria; and

a carrier for delivering said at least one lytic enzyme to a mouth, throat, or nasal passage.

58) The composition according to claim 57, wherein said bacteria being treated is selected from the group consisting of *Streptococcus pneumoniae* and *Hemophilus influenza*.

59) The composition according to claim 58, wherein said bacteria being treated is *Streptococcus pneumoniae*.

60) The composition according to claim 58, wherein said bacteria being treated is *Hemophilus influenza*.

61) The composition according to claim 57, wherein said carrier is selected from the group consisting of a candy, chewing gum, lozenge, troche, tablet, a powder, an aerosol, a liquid and a liquid spray.

- 62) The composition according to claim 57, wherein said composition further comprises a buffer that maintains pH of the composition at a range between about 4.0 and about 9.0.
- 63) The composition according to claim 62, wherein the buffer maintains the pH of the composition at the range between 5.5 and 7.5.
- 64) The composition according to claim 62, wherein said buffer comprises a reducing reagent.
- 65) The composition according to claim 64, wherein said reducing reagent is dithiothreitol.
- 66) The composition according to claim 62, wherein said buffer comprises a metal chelating reagent.
- 67) The composition according to claim 66, wherein said metal chelating reagent is ethylenediaminetetracetic disodium salt.
- 68) The composition according to claim 62, wherein said buffer is a citrate-phosphate buffer.
- 69) The composition according to claim 57, further comprising a bactericidal or bacteriostatic agent as a preservative.
- 70) The composition according to claim 57, wherein said lytic enzyme is lyophilized.
- 71) The composition according claim 57, wherein said at least one lytic enzyme is present in a concentration of about 100 to about 100,000 active

enzyme units per milliliter of fluid in the wet environment of the nasal or oral passages.

72) The composition according to claim 71, wherein said at least one lytic enzyme is present in a concentration of about 100 to about 10,000 active enzyme units per milliliter of fluid in the wet environment of the nasal or oral passages.

73) A method for the treatment of bacterial infections of the digestive tract, comprising administering to the digestive tract a composition comprising an effective amount of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria.

74) The method according to claim 73, further comprising delivering said lytic enzyme in a carrier suitable for delivering said lytic enzyme to the digestive tract.

75) The method for the treatment of bacterial infections according to claim 73, wherein said gram negative bacterial infections are caused by bacteria selected from the group consisting of *Listeria*, *Salmonella*, *E. coli*, and *Campylobacter*.

76) The method for the treatment of bacterial infections according to claim 73, wherein said carrier is selected from the group consisting of suppository enemas, syrups, and enteric coated pills.

77) A composition for treating for the treatment of bacterial infections of the digestive tract, comprising an effective amount of at least one lytic enzyme produced by said bacteria being infected with a bacteriophage specific for said bacteria, and

a carrier for delivering said lytic enzyme to the digestive tract.

78) The composition according to claim 77, wherein said bacteria to be treated are selected from the group consisting of *Listeria*, *Salmonella*, *E. coli*, and *Campylobacter*.

79) The composition according to claim 77, wherein said carrier for delivering said at least one lytic enzyme to the digestive tract is selected from the group consisting of suppository enemas, syrups, or enteric coated pills.

80) The composition of claim 77, wherein said composition further comprises a buffer that maintains pH of the composition at a range between about 4.0 and 9.0.

81) The composition according to claim 80, wherein the buffer maintains the pH of the composition at the range between 5.5 and 7.5.

82) The composition according to claim 80, wherein said buffer comprises a reducing reagent.

83) The composition according to claim 82, wherein said reducing reagent is dithiothreitol.

84) The composition according to claim 80, wherein said buffer comprises a metal chelating reagent.

85) The composition according to claim 84, wherein said metal chelating reagent is ethylenediaminetetracetic disodium salt.

86) The composition according to claim 80, wherein said buffer is a citrate-phosphate buffer.

- 87) The composition according to claim 77, further comprising a bactericidal or bacteriostatic agent as a preservative.
- 88) The composition according to claim 77, wherein said at least one lytic enzyme is lyophilized.
- 89) The composition according claim 77, wherein said at least one lytic enzyme is present in a concentration of about 100 to about 100,000 active enzyme units per milliliter of fluid in the wet environment of the digestive tract
- 90) The composition according to claim 89, wherein said at least one lytic enzyme is present in a concentration of about 100 to about 10,000 active enzyme units per milliliter of fluid in the wet environment of the digestive tract.
- 91) A composition for the therapeutic or prophylactic treatment of bacterial infections of burns and wounds of the skin, comprising:
an effective amount of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria; and
a carrier for delivering said at least one lytic enzyme to the skin.
- 92) The composition according to claim 91, wherein said carrier is a bandage.
- 93) The composition according to claim 91, further comprising using said composition in the prophylactic treatment of bacterial infections.
- 94) The composition according to claim 91, further comprising using said composition in the therapeutic treatment of bacterial infections.
- 95) The composition according to claim 91, wherein said bacteria being treated is *Pseudomonas*.

- 96) The composition according to claim 95, wherein said lytic enzyme is produced by the *Pseudomonas* bacteria being infected with a bacteriophage specific for the *Pseudomonas*.
- 97) The composition according to claim 86, wherein said bacteria being treated is *Staphylococcus*.
- 98) The composition according to claim 97, wherein said lytic enzyme is produced by the *Staphylococcus* bacteria being infected with a bacteriophage specific for the *Staphylococcus*.
- 99) The composition according to claim 91, wherein said bacterium being treated are *Staphylococcus* and *Pseudomonas*.
- 100) The composition according to claim 99, wherein said lytic enzymes are produced by the *Staphylococcus* bacteria being infected with a bacteriophage specific for the *Staphylococcus*, and *Pseudomonas* bacteria being infected with a bacteriophage specific for the *Pseudomonas*.
- 101) A method for the therapeutic or prophylactic treatment of bacterial infections of burns and wounds of the skin, comprising:
administering to an infected area of the skin a composition comprising an effective amount of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria..
- 102) The method according to claim 101, further comprising delivering said lytic enzyme in a carrier suitable for delivering said lytic enzyme to the skin.
- 103) The method according to claim 102, wherein said carrier is a bandage.

104) The method according to claim 101, further comprising using said composition in the prophylactic treatment of bacterial infections.

105) The method according to claim 101, further comprising using said composition in the therapeutic treatment of bacterial infections.

106) The method according to claim 101, wherein said bacteria being treated is *Pseudomonas*.

107) The method according to claim 106, wherein said lytic enzyme is produced by the *Pseudomonas* bacteria being infected with a bacteriophage specific for the *Pseudomonas*.

108) The method according to claim 101, wherein said bacteria being treated is *Staphylococcus*.

109) The method according to claim 108, wherein said lytic enzyme is produced by the *Staphylococcus* bacteria being infected with a bacteriophage specific for the *Staphylococcus*.

110) The method according to claim 101, wherein said bacterium being treated are *Staphylococcus* and *Pseudomonas*.

111) The method according to claim 110, wherein said lytic enzymes are produced by the *Staphylococcus* bacteria being infected with a bacteriophage specific for the *Staphylococcus*, and *Pseudomonas* bacteria being infected with a bacteriophage specific for the *Pseudomonas*.

112) A method for the prophylactic and therapeutic treatment of vaginal infections, comprising:

administering to the vagina composition comprising an effective amount of at least one lytic enzyme produced by said bacteria being infected with a bacteriophage specific for said bacteria.

113) The method according to claim 112, further comprising delivering said lytic enzyme in a carrier suitable for delivering said lytic enzyme to the vagina.

114) The method according to claim 113, wherein said carrier to be placed in the vagina.

115) The method according to claim 113, wherein said carrier is a tampon.

116) The method according to claim 113, wherein said carrier is a pad.

117) The method according to claim 113, wherein said carrier is a douche.

118) The method according to claim 112, wherein said lytic enzyme is specific for Group B *Streptococcus*.

119) A composition for the prophylactic and therapeutic treatment of treatment of vaginal infections, comprising:

an effective amount of at least one lytic enzyme produced by said bacteria being infected with a bacteriophage specific for said bacteria; and
a carrier for delivering said lytic enzyme to a vagina.

120) The composition according to claim 119, wherein said carrier is a tampon.

121) The composition according to claim 119, wherein said carrier is a douche.

122) The composition according to claim 119, wherein said carrier is a pad.

123) The composition according to claim 119, wherein said lytic enzyme is specific for Group B *Streptococcus*.

124) A method for the prophylactic and therapeutic treatment of eye infections, comprising:

administering to an eye a composition comprising an effective amount of at least one lytic enzyme produced by said bacteria being infected with a bacteriophage specific for said bacteria.

125) The method according to claim 124, further comprising delivering said lytic enzyme in a carrier suitable for delivering said lytic enzyme to the eye.

126) The method according to claim 124, wherein said bacteria being treated is *Hemophilus*.

127) The method according to claim 124, wherein said bacteria being treated is *Staphylococcus*.

128) The method according to claim 125, wherein the carrier is an eye drop solution.

129) The method according to claim 125, wherein the carrier is an eye wash solution.

130) The method according to claim 128, wherein said solution is an isotonic solution.

131) A composition for use in the therapeutic or prophylactic treatment of an eye infection,, comprising:

an effective amount of at least one lytic enzyme produced by bacteria being infected with a bacteriophage specific for said bacteria; and
a carrier for delivering said lytic enzyme to the eye.

132) The composition according to claim 131, wherein said bacteria being treated is *Hemophilus*.

133) The composition according to claim 131, wherein said bacteria being treated is *Staphylococcus*.

134) The composition according to claim 131, wherein said carrier is an isotonic solution.

135) The composition according to claim 134, wherein said isotonic solution is in an eye drop dispenser.

136) A method for the prophylactic or therapeutic treatment of dermatological infections comprising:

topically applying to an infected area of the skin a composition comprising an effective amount of at least one lytic enzyme produced by bacteria infected with a bacteriophage specific for said bacteria..

137) The method according to claim 136, further comprising delivering said composition in a pharmaceutically acceptable carrier.

138) The method according to claim 137, wherein said carrier is selected from the group consisting of an aqueous liquid, an alcohol base, a water soluble gel, a lotion, an ointment, a nonaqueous liquid base, a mineral oil base, a blend of mineral oil and petrolatum, lanolin, liposomes, hydrophilic gelling agents, cross-linked acrylic acid polymers (carbomer), cellulose polymers, hydroxy ethyl

cellulose, cellulose gum, MVE/MA decadiene crosspolymers, PVM/MA copolymers, and any combinations thereof.

139) The method according to claim 136, wherein the form in which the composition is delivered is selected from the group consisting of a spray, a smear, a time release patch, a liquid absorbed wipe, and any combinations thereof.

140) The method according to claim 136, wherein the lytic enzyme is in an environment having a pH which allows for activity of said lysin enzyme.

141) The method according to claim 140, wherein said composition further comprises a buffer that maintains pH of the composition at a range between about 4.0 and about 9.0.

142) The method according to claim 141, wherein said buffer maintains the pH of the composition at the range of between about 5.5 and about 7.5.

143) The method according to claim 141, wherein said buffer comprises a reducing agent.

144) The method according to claim 143, wherein said reducing agent is dithiothreitol.

145) The method according to claim 131, wherein said composition further comprises a mild surfactant in an amount effective to potentiate effects of the lytic enzyme.

146) The method according to claim 131, wherein the composition further comprises at least one complementary agent which potentiates the bactericidal

activity of the lytic enzyme, said complementary agent being selected from the group consisting of penicillin, synthetic penicillins bacitracin, methicillin, cephalosporin, polymyxin, cefaclor, Cefadroxil, cefamandole nafate, cefazolin, cefixime, cefmetazole, cefonoid, cefoperazone, ceforanide, cefotanme, cefotaxime, cefotetan, cefoxitin, cefpodoxime proxetil, ceftazidime, ceftizoxime, ceftriaxone, ceftriaxone moxalactam, cefuroxime, cephalixin, cephalosporin C, cephalosporin C sodium salt, cephalothin, cephalothin sodium salt, cephapirin, cephradine, cefuroximeaxetil, dihydratecephalothin, moxalactam, loracarbef, nafate and chelating agents in an amount effective to synergistically enhance effects of the lytic enzyme.

147) The method according to claim 136, wherein the composition further comprises lysostaphin for the treatment of any *Staphylococcus aureus* bacteria.

148) The method according to claim 136, wherein said lytic enzyme is present in an amount ranging from about 100 to about 500,000 units per milliliter.

149) A composition for the treatment of dermatological *Streptococcus* infections comprising:

an effective amount of at least one lytic enzyme produced by bacteria infected with a bacteriophage specific for said bacteria and a carrier for topical application of the at least one lytic enzyme.

150) The composition according to claim 149, wherein said carrier is selected from the group consisting of an aqueous liquid, an alcohol base, a water soluble gel, a lotion, an ointment, a nonaqueous liquid base, a mineral oil base, a blend of mineral oil and petrolatum, lanolin, liposomes, hydrophilic gelling agents, cross-linked acrylic acid polymers (carbomer), cellulose polymers, hydroxy ethyl cellulose, cellulose gum, MVE/MA decadiene crosspolymers, PVM/MA copolymers, and any combinations thereof.

151) The composition according to claim 150, wherein said composition is in the form selected from the group consisting of a spray, a smear, a time release patch, a liquid absorbed wipe, and any combinations thereof.

152) The composition according to claim 149, wherein the at least one lytic enzyme is in an environment having a pH which allows for activity of said lytic enzyme.

153) The composition according to claim 149, wherein said composition further comprises a buffer that maintains pH of the composition at a range between about 4.0 and about 9.0.

154) The composition according to claim 153, wherein said buffer maintains the pH of the composition at the range of between about 5.5 and about 7.5.

155) The composition according to claim 153, wherein said buffer comprises a reducing agent.

156) The composition according to claim 149, wherein said reducing agent is dithiothreitol.

157) The composition according to claim 153, wherein said buffer comprises a metal chelating reagent.

158) The composition according to claim 149, further comprising a bactericidal or bacteriostatic agent as a preservative.

159) The composition according to claim 149, further comprising a surfactant in an amount effective to potentiate a therapeutic effect of the composition.

160) The composition according to claim 149, wherein the composition further comprises at least one complementary agent which potentiates the bactericidal activity of the lytic enzyme, said complementary agent being selected from the group consisting of penicillin, synthetic penicillins bacitracin, methicillin, cephalosporin, polymyxin, cefaclor. Cefadroxil, cefamandole nafate, cefazolin, cefixime, cefmetazole, cefonoid, cefoperazone, ceforanide, cefotanme, cefotaxime, cefotetan, cefoxitin, cefpodoxime proxetil, ceftazidime, ceftizoxime, ceftriaxone, cefriaxone moxalactam, cefuroxime, cephalixin, cephalosporin C, cephalosporin C sodium salt, cephalothin, cephalothin sodium salt, cephapirin, cephradine, cefuroximeaxetil, dihydratecephalothin, moxalactam, loracarbef. mafate chelating agents, and combinations thereof in an amount effective to synergistically enhance the therapeutic effect of the lytic enzyme.

161) The composition according to claim 149, wherein the composition further comprises lysostaphin for the treatment of any *Staphylococcus aureus* bacteria.

162) The composition according to claim 149, wherein the composition further comprises lysozyme.

163) The composition according to claim 149, further comprising at least one emulsifier.

164) The composition according to claim 149, further comprising at least one antioxidant.

165) The composition according to claim 149, further comprising at least one sunscreen.

166) The composition according to claim 149, further comprising at least one preservative.

167) The composition according to claim 149, further comprising at least one anti-inflammatory agent.

168) A composition for the therapeutic or prophylactic treatment of bacterial infections of the upper respiratory system, comprising an effective amount of at least one lytic enzyme produced by bacteria infected with a bacteriophage specific for that bacteria and a pharmaceutically acceptable carrier in an inhaler allowing for the administration of the at least one lytic enzyme to the bronchial tubes and lungs.

169) The composition according to claim 168, wherein said composition is for the therapeutic treatment of bacterial infections of the upper respiratory system.

170) The composition according to claim 168, wherein said composition is for the prophylactic treatment of bacterial infections of the upper respiratory system.

171) A composition for the therapeutic or prophylactic treatment of bacterial infections of the mouth or teeth, comprising an effective amount of at least one lytic enzyme produced by bacteria infected with a bacteriophage specific for that bacteria and a pharmaceutically acceptable carrier for topical application of the at least one lytic enzyme.

172) The composition according to claim 171, wherein in said composition is for the treatment of dental caries.

173) The composition according to claim 172, wherein said composition is used for the prophylactic treatment of dental caries.

174) The composition according to claim 172, wherein said composition is used for the therapeutic treatment of dental caries.

175) The composition according to claim 171, wherein said carrier is toothpaste.

176) The composition according to claim 171, wherein said carrier is an oral wash.

177) The composition according to claim 171, wherein said carrier is a chewing gum.

178) The composition according to claim 171, wherein said carrier is a lozenge.

179) The composition according to claim 171, wherein said bacteria being treated is *Streptococcus mutans*.

180) The composition according to claim 171, wherein said lytic enzyme is present in an amount ranging from about 100 to about 500,000 units per milliliter.

181) The composition according to claim 180, wherein said lytic enzyme is present in an amount ranging from about 10,000 to about 100,000 units per milliliter.

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 32876/120	FOR FURTHER ACTION SEE Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/US 00/ 25093	International filing date (day/month/year) 14/09/2000	(Earliest) Priority Date (day/month/year) 14/09/1999
Applicant NEW HORIZONS DIAGNOSTICS CORPORATION et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 8 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.



as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.



None of the figures.

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K38/48 A61P31/02 A61P31/04 A61P15/02 A61P17/02
 A61P27/02 A61K38/46

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 02351 A (CIBA GEIGY AG ;UNIV CAMBRIDGE TECH (GB); LUZIO JOHN PAUL (GB); BRY) 23 January 1997 (1997-01-23)	1,6-38, 43-62, 66-75, 79-82, 86-91, 95-101, 105-112, 116-122, 126-130, 134-140, 144-148, 152-176, 180-182, 186-194
Y	abstract page 6, paragraph 4 -page 7, paragraph 2 claims 20-22,24 --- -/--	1-194

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *8* document member of the same patent family

Date of the actual completion of the international search

17 April 2001

Date of mailing of the international search report

04/05/2001

Name and mailing address of the ISA

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Authorized officer

Noë, V

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 07329 A (UNIV MARYLAND) 14 March 1996 (1996-03-14) abstract page 1, line 2 - line 8 page 5, line 17 - line 20 page 12, line 20 - line 29	1,6,16, 18,20, 27-29, 31,33, 34,36, 37,182, 186-194
Y	claims 1-5,12,13,20 ---	1-194
X	EP 0 510 907 A (AGRICULTURAL & FOOD RES) 28 October 1992 (1992-10-28)	1,6-38, 43-62, 66-75, 79-82, 86-91, 95-101, 105-112, 116-122, 126-130, 134-140, 144-148, 152-176, 180-182, 186-194
Y	abstract page 2, line 12 - line 13 page 2, line 26 - line 30 page 2, line 49 - line 51 page 3, line 4 - line 6 page 6 ---	1-194
Y	SHEEHAN M M ET AL: "THE LYTIC ENZYME OF THE PNEUMOCOCCAL PHAGE DP-1: A CHIMERIC LYSIN OF INTERGENERIC ORIGIN" MOLECULAR MICROBIOLOGY,GB,BLACKWELL SCIENTIFIC, OXFORD, vol. 25, no. 4, 1997, pages 717-725, XP000922620 ISSN: 0950-382X abstract page 722, column 1, last paragraph -page 723, column 2, paragraph 1 ---	1-194
Y	WITTE A ET AL: "Characterization of Escherichia coli lysis using a family of chimeric E-L genes." FEMS MICROBIOLOGY LETTERS, vol. 164, no. 1, 1 July 1998 (1998-07-01), pages 159-167, XP000992671 ISSN: 0378-1097 cited in the application the whole document ---	1-194

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MARTIN ANA C ET AL: "Functional analysis of the two-gene lysis system of the pneumococcal phage Cp-1 in homologous and heterologous host cells." JOURNAL OF BACTERIOLOGY, vol. 180, no. 2, January 1998 (1998-01), pages 210-217, XP000992652 ISSN: 0021-9193 abstract page 212, column 1, last paragraph -page 216, paragraph 1 ---	1-194
Y	OKI MASAYA ET AL: "Functional and structural features of the holin HOL protein of the Lactobacillus plantarum phage phi-gle: Analysis in Escherichia coli system." GENE (AMSTERDAM), vol. 197, no. 1-2, 1997, pages 137-145, XP004126411 ISSN: 0378-1119 abstract page 137, column 2, paragraph 1 page 139, column 1, paragraph 1 - paragraph 3 page 139, column 2, paragraph 2 page 142, column 1, last paragraph -column 2 page 144, column 2, last paragraph -page 145 ---	1-194
A	WO 99 04809 A (AMBI INC) 4 February 1999 (1999-02-04) abstract page 1 page 4, line 2 - line 22 page 8, line 28 -page 9, line 2 examples 1-5 ---	5,42, 158,159, 169,170
T	NELSON D ET AL: "Prevention and elimination of upper respiratory colonization of mice by group A streptococci by using a bacteriophage lytic enzyme" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES , vol. 98, no. 7, 27 March 2001 (2001-03-27), pages 4107-4112, XP002165225 the whole document -----	1-194

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FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

6. Claims: 1-6,19-37,75-90 (partially) and 13 (completely)

Method and compositions for prophylactic or therapeutic treatment of E. Coli infections comprising administering to the site of infection an effective amount of at least one lytic enzyme produced by a bacteria infected with a bacteriophage.

7. Claims: 1-6,19-37,75-90 (partially) and 14 (completely)

Method and compositions for prophylactic or therapeutic treatment of Campylobacter infections comprising administering to the site of infection an effective amount of at least one lytic enzyme produced by a bacteria infected with a bacteriophage.

8. Claims: 1-6, 19-37,38-43,46-57,
62-65 (partially) and 17 (completely)

Method and compositions for prophylactic or therapeutic treatment of Mycobacterium tuberculosis infections comprising administering to the site of infection an effective amount of at least one lytic enzyme produced by a bacteria infected with a bacteriophage.

9. Claims: 1-6, 19-37,91-97,100-108,111,130-134,137-143,
146-147 (partially) and 99,110,136,145,148-175

Method and compositions for prophylactic or therapeutic treatment of Staphylococcus infections comprising administering to the site of infection an effective amount of at least one lytic enzyme produced by a bacteria infected with a bacteriophage.

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1,3,4 relate to a method using a compound defined by reference to a desirable characteristic or property, namely a shuffled or a chimeric lytic enzyme and a shuffled or chimeric holin enzyme.

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to an activity to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds described on page 5, line 6-20 for chimeric enzymes. The term shuffled as defined on page 5, line 1-3 does not allow a meaningful search.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/25093

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-61, 75-81, 101-121, 130-139, 148-160 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

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